Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma

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Abstract: EGFR amplification (EGFRamp), the combination of gain of chromosome 7 and loss of chromosome 10 (7+/10-), and TERT promoter mutation (pTERTmut) are alterations frequently observed in adult IDH-wild-type (IDHwt) glioblastoma (GBM). In the absence of endothelial proliferation and/or necrosis, these alterations currently are considered to serve as a surrogate for upgrading IDHwt diffuse or anaplastic astrocytoma to GBM. Here, we set out to determine the distribution of EGFRamp, 7+/10-, and pTERTmut by analyzing high-resolution copy-number profiles and next-generation sequencing data of primary brain tumors. In addition, we addressed the question whether combinations of partial gains on chromosome 7 and partial losses on chromosome 10 exhibited a diagnostic and prognostic value similar to that of complete 7+/10-. Several such combinations proved relevant and were combined as the 7/10 signature. Our results demonstrate that EGFRamp and the 7/10 signature are closely associated with IDHwt GBM. In contrast, pTERTmut is less specific for IDHwt GBM. We conclude that, in the absence of endothelial proliferation and/or necrosis, the detection of EGFRamp is a very strong surrogate marker for the diagnosis of GBM in IDHwt diffuse astrocytic tumors. The 7/10 signature is also a strong surrogate marker. However, care should be taken to exclude pleomorphic xanthoastrocytoma. pTERTmut is less restricted to this entity and needs companion analysis by other molecular markers to serve as a surrogate for diagnosing IDHwt GBM. A combination of any two of EGFRamp, the 7/10 signature and pTERTmut, is highly specific for IDHwt GBM and the combination of all three alterations is frequent and exclusively seen in IDHwt GBM.

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Distribution of *EGFR* amplification, combined 7gain/10loss and *TERT* promoter mutation in brain tumors and their potential for the reclassification of *IDH*wt astrocytoma to glioblastoma

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Abstract

EGFR amplification (*EGFR*amp), the combination of gain of chromosome 7 and loss of chromosome 10 (7+/10-) and *TERT* promoter mutations (p*TERT*mut) are alterations frequently observed in glioblastoma (GBM). In absence of endothelial proliferations and/or necrosis, these alterations currently are considered to serve as a surrogate for upgrading *IDH* wildtype (*IDH*wt) astrocytoma to GBM. Here we set out to determine the distribution of *EGFR*amp, 7+/10- and p*TERT*mut by analyzing intracranial tumors with high resolution copy number profiles (CNP) and sequencing data available. In addition, we addressed the question whether combinations of partial gains on 7 and losses on 10 exhibited a diagnostic and prognostic value similar to that of complete 7+/10-. Several such combinations proved relevant and were combined and designated 7/10 signature. Our results demonstrate that *EGFR*amp and 7/10 are closely associated with GBM. In contrast, p*TERT*mut is less specific for GBM. We conclude that in the absence of endothelial proliferations and/or necrosis the detection of *EGFR*amp is a very strong surrogate marker for the diagnosis of GBM in diffuse astrocytic tumors. The 7/10 signature is also a strong surrogate marker. However, care should be taken to exclude pleomorphic xanthoastrocytoma. p*TERT*mut is less restricted to this entity and needs companion analysis by other molecular markers in order to serve as a surrogate for diagnosing GBM. A combination of any two of *EGFR*amp, 7/10 and p*TERT*mut is highly specific for GBM and the combination of all three alterations is frequent and exclusively seen in GBM.

Keywords

EGFR amplification, 7gain, 10loss, 7+/10-, , 7+/10q-, *TERT*, promoter, glioblastoma, astrocytoma, pleomorphic xanthoastrocytoma

Introduction

~~The so called~~ *IDH* wildtype (*IDH*wt) astrocytoma comprises different tumors with the majority exhibiting molecular lesions and survival characteristics of glioblastoma (GBM). A pressing question is whether *EGFR* amplification, the presence of a combined gain on chromosome 7 and loss on chromosome 10 and *TERT* promoter mutation can serve as surrogate marker for upgrading *IDH*wt astrocytoma, by definition lacking necrosis, to glioblastoma.

Kommentiert [WM1]: When do you abbreviate, when not?

The combination of gains on chromosome 7 and losses on chromosome 10 are characteristic molecular alterations in glioblastoma (GBM). Recent studies have demonstrated that astrocytic tumors not fulfilling the morphological criteria for GBM but exhibiting typical molecular features of GBM exhibit a clinical course similar to that of morphologically unequivocal GBM [5,11,24,30,31]. Therefore, the presence of the combination of 7gain and 10 loss is considered as molecular marker for GBM. Little is known about the relevance of assessing individual arms of chromosomes 7 and 10, i.e. does gain of either arm of 7 and loss of either arm of 10 suffice or is gain of both arms on 7 and loss of both arms on 10 required. Gain of chromosome 7 and loss of chromosome 10 in GBM haves ~~s~~ been initially detected by cytogenetic analyses [25] [4]. The typical constellation found in GBM cell lines was trisomy of chromosomes 7 and monosomy of chromosome 10 most likely as a result of error in mitotic disjunction.

EGFR amplification (*EGFR*amp) in GBM has been initially observed by molecular and cytogenetic analyses [18,33] [3,9]. Roughly half of the GBM exhibit a strong amplification of this gene mostly due to the presence of numerous double minutes, the extra chromosomal elements containing additional *EGFR* copies [28].

Telomerase reverse transcriptase (*TERT*) encodes the catalytic subunit of the telomerase complex. *TERT* promoter mutation (p*TERT*mut) initially has been detected in melanoma [12,13]. Subsequent investigations also revealed high frequencies of p*TERT*mut in GBM and in oligodendroglioma. [1,14,15,19,21,32], ~~-~~ and their potential use for subgrouping of glioma and in a diagnostic setting [2,7,8,22].

Recent technology development allows generating high resolution copy number profiles (CNP) of the human chromosomes in tumor tissue based on next generation sequencing or array data. We set out to address the distribution of *EGFR*amp, 7+/10- and p*TERT*mut in human brain tumors based on CNP, generated from DNA methylation array data sets and from next generation sequencing data. Furthermore, we explored the prognostic association of these alterations in brain tumors diagnosed as *IDH*wt astrocytoma.

Material and Methods

Patient cohorts

The present analyses are based on data from three cohorts available for analysis at the Department. of Neuropathology of the University Heidelberg. From all patients in the three cohorts 450K/850K methylation array data are available. All patients have received a methylation based diagnosis as previously described [6].

Cohort 1 contains 2.417 patients for whom next generation panel sequencing with a panel including the *TERT* gene and its promoter has been performed. Cohort 1 was employed for determining the distribution of p*TERT*mut, *EGFR*amp and 7+/10- in human brain tumors. Only one tumor per patient was included in cohort 1. Table 1 lists all tumors included in cohort 1 sorted by methylation based diagnosis.

Cohort 2 including 10.826 brain tumor patients with have been analyzed using the Illumina 450K or 850K platforms is the basis of the distribution of *EGFR*amp and 7+/10- in human brain tumors. Only one tumor per patient was included in cohort 2. Supplementary table 1 lists all tumors included in cohort 2 sorted by methylation based diagnosis [6] and respective number of patients. Cohort 2 encompasses all patients from cohort 1. Details on methylation classes can be obtained from www.molecularneuropathology.org.

Cohort 3 contains 939 patients from cohort 2 with survival data available. In contrast to cohorts 1 and 2 the histological diagnosis according to WHO 2016 [20] including the diagnosis of *IDH*wt astrocytoma was basis for survival analysis. Cohort 3 does not include any patients diagnosed as glioblastoma. There is a bias in cohort 3 because survival data have been acquired for specific tumor entities in previous studies.

Generation and scoring of CNPs and mutations

Methylation data were generated using the Illumina 450K or 850K/EPIC platforms as previously described [6].The copy number variation plots were generated from the same raw data using the 'conumee' R package in Bioconductor (http://www.bioconductor.org packages/release/bioc/html / conumee.html). Figure 1 shows representative CNPs. Automated assessment of copy-number changes was performed using the results from conumee after additional baseline correction. EGFRamp was called amplified if the respective probes exhibited an intensity higher than 0.6 on a log2-scale.

Panel sequencing was performed a previously reported [26]. pTERTmut was scored if 10 or more reads were detected with a minimum of 10% of the reads showing either of the two TERT promoter mutations mutation.

Statistics

All patient sets were retrospectively compiled. The size of the respective sets was determined by availability of data and not by a power calculation. OS times were analyzed by the Kaplan-Meier method and compared with a log-rank test. P values less than 0.05 were considered significant. Software R version 3.4 and packages survival and party were employed for analysis

Results and Discussion

Rationale and procedure

We aimed at contributing to three questions: First, what is the incidence of combined 7/10 alterations, *EGFR*amp and p*TERT*mut in human brain tumors. Second, are these three alterations suitable surrogate markers for diagnosing GBM in the absence of necrosis and microvascular proliferation? and third, which variants of 7/10 alterations might be employed. The first two questions have been addressed by analyzing a series of 2,417 tumors (cohort 1) with both *TERT* promoter status and complete copy number profiles being available (table 1). Question three was addressed by analyzing an extended set of 10,826 patients (cohort 2) with complete copy number profiles available (supplementary table 1) and analyzing a subset thereof including 939 (cohort 3) patients with overall survival data available.

For all series, both, a classical histopathological and a methylation based diagnosis was available. Current WHO GBM includes the H3-G34mutant GBM, while the H3-K27 GBM and the *IDH* mutant (*IDH*mut) GBM have been separated from the group of *IDHwt* GBM. For all questions addressing association of the markers interrogated with survival, we adhered to the current WHO definition of GBM (all analyses involving cohort 3). For determination of frequencies we used the methylation based diagnosis as this is highly standardized and, therefore, more suitable for this type of question.

Defining 7/10 status

An open question is whether 7+/10- is prognostically relevant only if both arms each chromosome are affected or if losses of only one arm per chromosome suffice. Further, how much of each chromosomal arm needs to be affected to score either a gain or a loss? This question is of special interest in light of many data being FISH analysis based and, therefore, not providing representative information at all. Our approach is array platform based thereby covering the entire chromosomal arms. We selected two different thresholds with one being 50% and the other 80% of chromosomal representation being gained or lost for calling the respective alteration in cohort 1. Supplementary table 1 provides an overview of 7+ and 10- combinations using both thresholds. In the predominant GBM subgroup characterized by gain of entire 7 and loss of entire 10, 1185 patients (75% of all GBM) scored positive with the 80% and 1265 patients (81% of all GBM) with the 50% threshold (supplementary table 1). We therefore went on using the 50% threshold for all subsequent analyses. Of 9 possible combinations exhibiting both, gain of at least one arm on 7 and loss of at least one arm on 10, 7+/10- represents the most frequent (1265/1598; 79%) constellation followed by 7+/10q- (87/1598; 5%), by 7p+/10- (74/1598; 5%) and by 7q+/10- (70/1598; 4%) (supplementary table1).

Next we analyzed which variants of gains and losses were associated with unfavorable clinical outcome. To this end we analyzed the respective combinations in patients from cohort 3.

Of the nine possibilities for 7gain and 10loss combinations we encountered 7+/10- (n=97), 7q+/10- (n=12), 7+/10q- (n=9), 7q+/10q- (n=7), 7p+/10- (n=3), both 7+/10p- and 7q+/10p- (n=1), and both 7p+/10p and 7p+/10q- (n=0) in patients from cohort 3. Survival analysis was performed of patients exhibiting the combinations 7+/10- (n=97), 7q+/10- (n=12), 7+/10q- (n=9), 7q+/10q- (n=7) and

7p+/10- (n=3). Three combinations, 7+/10- , 7q+/10- , 7+/10q- associated with poor survival similar to that of patients with GBM (figure 2a). We therefore defined all patients with 7+/10-, 7+/10q- and 7q+/10- as carrying the prognostic 7/10 signature. Noteworthy, methylation based classification identified a substantial number of patients in cohort 3 without a 7/10 signature but with typical survival characteristics of GBM (figure 2b)demonstrating the power of this method.

Distribution of *EGFR*amp, 7/10 signature and *TERT* in human brain tumors

Comparison of the distribution of all three parameters was performed using cohort 2 although for the distribution of *EGFR*amp and 7/10 signature a higher resolution can be obtained from cohort 1.
Frequent p*TERT*mut was observed in 363/544 (67%) GBM, in 95/120 (79%) oligodendroglioma, in 12/17 (71%) melanoma, in 19/42 (45%) medulloblastoma methylation subclass SHH A and in 7/34 (21%) pleomorphic xanthoastrocytoma (PXA). Numbers for p*TERT*mut in entities with low mutation frequencies or entities with only few tumors analyzed are given in the table 1. Of the three parameters, *TERT* exhibited highest sensitivity (67%) but lowest specificity (89%) for glioblastoma (table 2).
The 7/10 signature was more specific for GBM being seen in 323/544 (59%) GBM, in 9/140 (6%) high grade *IDH*mut astrocytoma including *IDH*mut GBM and in 5/54 (9%) medulloblastoma of methylation subgroup 4 (table 1). Sensitivity was 59% and specificity was 98% (table 2).
*EGFR*amp was observed in 196/544 (36%) GBM showing lowest sensitivity (36%) but highest specificity (100%) for GBM (table 2).
On the entire scale p*TERT*mut (562/2417) is more frequent than *EGFR*amp (199/2417) or 7/10 (361/2417). The combinations of p*TERT*mut – *EGFR*amp (28 cases), p*TERT*mut – 7/10 (146 cases) and *EGFR*amp – 7/10 (30 cases) were strongly associated with GBM and the triple combination of p*TERT*mut – 7+/10- - *EGFR*amp (124 cases) was exclusively seen in GBM (table 1).The sensitivity of any combination of double or triple positives was 58% and the specificity was 99% (table 2).

Distribution of *EGFR*amp, 7/10 signature and p*TERT*mut **in GBM methylation classes**

Subdivision of *IDH*wt GBM by methylation based classification results in 7 subgroups. These subgroups exhibit striking differences in the frequencies of the tree parameters interrogated. Tumors of the methylation class glioblastoma, *IDH*wt, H3.3 G34 mutant (n=17) did not exhibit p*TERT*mut or *EGFR*amp. The 7/10 signature was observed only in four H3.3 G34 mutant GBM . This finding is quite similar to that in H3.3 K27 mutant GBM and arguing for separating the H3.3 G34 mutant GBM from the *IDH*wt GBM, too. Tumors of the methylation class glioblastoma, *IDH*wt, subclass MYCN (n=22) exhibited p*TERT*mut and *EGFR*amp in less than quarter of all cases and the 7/10 signature only in a single tumor. Methylation class glioblastoma, *IDH*wt, subclass RTK I (n=71) presented with p*TERT*mut in 55 cases (77%), 18 (25%) times *EGFR*amp and 46 (65%) times the 7/10 signature. Methylation class glioblastoma, *IDH*wt, subclass RTK II (n=203) constituting the most frequent GBM subgroup presented with p*TERT*mut in 166 cases (83%), 128 (63%) times *EGFR*amp and 160 (79%) times the 7/10 signature. The methylation class glioblastoma, *IDH*wt, subclass RTK III (n=23) predominantly encountered in young patients exhibited 11 times (48%) p*TERT*mut, 8 times (35%) *EGFR*amp and 3 times (13%) 7/10. The methylation class glioblastoma, *IDH*wt, subclass mesenchymal (n=157) is frequent and presented with 123 (78%) p*TERT*mut, 37 (24%) *EGFR*amp and 109 (69%) 7/10

signatures. And finally tumors falling into the methylation class glioblastoma, *IDH*wt, subclass midline (n=51) which is a yet poorly characterized group of tumors with morphology and survival characteristics comparable to that of GBM [24] exhibited p*TERT*mut in 4 (8%) and *EGFR*amp or a 7/10 signature in neither cases.
Clearly as morphological GBM comprises all these methylation subgroups, the sensitivity of *EGFR*amp, 7/10 signature and p*TERT*mut based grading is compromised by the low or absent prevalence in some of these subgroups.

Single use of one of the three parameters *EGFR*amp, 7/10 signature and p*TERT*mut

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A single molecular marker for diagnosing GBM in absence of necrosis would be a major contribution to daily routine diagnostics. While *EGFR*amp, p*TERT*mut or 7/10 signature are very good candidates, single use is not warranted. p*TERT*mut obviously needs accompanying analysis of *IDH*1 and *IDH*2 in order to exclude oligodendroglioma and astrocytoma. Also the rare adult medulloblastoma [23] and anaplastic meningioma [10,27] as well as SFT/Hemangiopericytoma frequently carry p*TERT*mut and need to be distinguished by appropriate testing. The presence of p*TERT*mut in PXA [16] should be addressed by testing for the *BRAF*V600E mutation typical for the latter [29]. However, an overlap with the rare epithelioid glioblastoma cannot be ruled out [17]. *EGFR*amp has highest specificity and *IDH* testing can separate the rare occurrences in *IDH* mutant glioma. The 7/10 signature also needs additional testing for *IDH* to separate for anaplastic diffuse glioma and for BRAF for its occasional occurrence in PXA (table 1).

Conclusions

Our data supports the single use of the molecular genetic markers *EGFR*amp, p*TERT*mut or 7/10 signature as strong markers for the reclassification of *IDH*wt astrocytoma to GBM pending additional molecular tests. The combination of a positive finding for any two of the three parameters is highly specific for and the combination of all three parameters is exclusively seen in GBM.

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Figure legends

Table 1

Classifier prediction, *EGFR*, 7/10 and *TERT* status in cohort 1. Full text for abbreviated methylation classes is provided in supplementary table 1. A characterization of methylation classes by Classifier prediction can be obtained from www.molecularneuropathology.org.

Table 2

Sensitivity and specificity of p*TERT*mut, *EGFR*amp and 7/10 signature as single parameters and in combination for 544 GBM in a series of 2417 brain tumors.

Figure 1

Representative CNP-plots: a) GBM with 7+/10- and *EGFR*amp; b) GBM with 7+/10q-; c) PXA with 7+/10-

Figure 2.

a) OS in 939 patients (cohort 3) which have been diagnosed as IDHwt glioma, excluding glioblastoma, stratified for different combinations of alterations of chromosomes 7 and 10. Of all possible combinations with losses on chromosomes 7 and 10, only 7+/10-, 7+/10q-,7p+10-, 7q+/10q- and 7q+/10- were represented more than 3 times. Survival of glioma patients with 7+/10-, 7+/10q- and 7q+/10- was significantly worse than that of patients without these alterations.
b) OS in167 patients from cohort 3 which in addition have received the classifier diagnosis GBM. Methylation based classification identifies 52 additional patients without a 7/10 signature. Black graph is a reference series of additional 261 patients diagnosed as GBM by both, histology and methylation based classification.

Supplementary table 1

Distribution of *EGFR*amp and status of chromosomes 7 and 10 in 10.826 tumors. Chromosome 7 and 10 status is given for two different thresholds requiring loss >50% or >80% of the respective arms. Combinations not qualifying for any form of a combined 7gain and 10loss are indicated by print in grey.

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Table 1

Methylation class abbreviation	n total	n *TERT*	n *EGFR*	n 7/10	triple wt	single 7/10	single EGFR	single *TERT*	double 7/10 + EGFR	double 7/10 + *TERT*	double EGFR + *TERT*	triple 7/10 + EGFR + *TERT*
PXA	34	7	0	2	26	1	0	6	0	1	0	0
EFT_CIC	3	0	0	0	3	0	0	0	0	0	0	0
HGNET_BCOR	9	0	0	0	9	0	0	0	0	0	0	0
HGNET_MN1	6	0	0	0	6	0	0	0	0	0	0	0
CNS_NB_FOXR2	1	0	0	0	1	0	0	0	0	0	0	0
EWS	5	1	0	0	4	0	0	1	0	0	0	0
O_IDH	120	95	0	1	25	0	0	94	0	1	0	0
A_IDH	154	5	0	1	148	1	0	5	0	0	0	0
A_IDH_HG	140	11	1	9	121	7	1	9	0	2	0	0
ANA_PA	75	2	0	1	72	1	0	2	0	0	0	0
ATRT_MYC	4	0	0	0	4	0	0	0	0	0	0	0
ATRT_SHH	7	0	0	0	7	0	0	0	0	0	0	0
ATRT_TYR	6	0	0	0	6	0	0	0	0	0	0	0
CN	18	0	0	0	18	0	0	0	0	0	0	0
LIPN	2	0	0	0	2	0	0	0	0	0	0	0
CHGL	1	0	0	0	1	0	0	0	0	0	0	0
CHORDM	4	1	1	2	2	0	0	0	1	1	0	0
CPH_ADM	2	0	0	0	2	0	0	0	0	0	0	0
CPH_PAP	4	0	0	0	4	0	0	0	0	0	0	0
DLGNT	9	0	0	0	9	0	0	0	0	0	0	0
DMG_K27	63	0	0	0	63	0	0	0	0	0	0	0
ETMR	25	0	0	0	25	0	0	0	0	0	0	0
EPN_RELA	14	0	0	0	14	0	0	0	0	0	0	0
EPN_YAP	1	0	0	0	1	0	0	0	0	0	0	0
EPN_MPE	15	0	0	1	14	1	0	0	0	0	0	0
EPN_PF_A	34	0	0	2	32	2	0	0	0	0	0	0
EPN_PF_B	6	1	0	0	5	0	0	1	0	0	0	0
EPN_SPINE	4	0	0	0	4	0	0	0	0	0	0	0
ENB_A	3	0	0	1	2	1	0	0	0	0	0	0
ENB_B	2	0	0	0	2	0	0	0	0	0	0	0
GBM_G34	17	0	0	4	13	4	0	0	0	0	0	0
GBM_MYCN	22	4	5	1	14	0	4	2	0	1	1	0
GBM_RTK_I	71	55	18	46	4	8	2	13	2	28	6	8
GBM_RTK_II	203	166	128	160	6	7	4	21	20	41	12	92
GBM_RTK_III	23	11	8	3	7	0	4	6	1	2	3	0
GBM_MES	157	123	37	109	12	15	1	29	6	64	6	24
GBM_MID	51	4	0	0	47	0	0	4	0	0	0	0
HMB	4	0	0	0	4	0	0	0	0	0	0	0
IHG	7	1	0	0	6	0	0	1	0	0	0	0
LGG_MYB	14	0	0	0	14	0	0	0	0	0	0	0
LGG_DIG_DIA	3	0	0	0	3	0	0	0	0	0	0	0
LGG_DNT	19	0	0	0	19	0	0	0	0	0	0	0
LGG_GG	12	3	0	2	9	0	0	1	0	2	0	0
LGG_RGNT	8	0	0	0	8	0	0	0	0	0	0	0
LGG_PA_GG_ST	30	1	0	1	29	0	0	0	0	1	0	0
LGG_PA_MID	34	0	0	0	34	0	0	0	0	0	0	0
LGG_PA_PF	78	0	0	0	78	0	0	0	0	0	0	0
LGG_SEGA	13	0	0	0	13	0	0	0	0	0	0	0
LYMPHO	13	0	0	0	13	0	0	0	0	0	0	0
MB_WNT	34	2	0	0	32	0	0	2	0	0	0	0
MB_SHH_CHL_AD	42	19	0	0	23	0	0	19	0	0	0	0
MB_SHH_INF	47	1	0	0	46	0	0	1	0	0	0	0
MB_G3	33	0	0	1	32	1	0	0	0	0	0	0
MB_G4	54	4	0	5	45	5	0	4	0	0	0	0
MELCYT	17	0	0	0	17	0	0	0	0	0	0	0
MELAN	17	12	0	1	5	0	0	11	0	1	0	0
SCHW_MEL	8	1	0	1	6	1	0	1	0	0	0	0
MNG	476	22	0	2	453	1	0	21	0	1	0	0
PTPR_A	4	0	0	0	4	0	0	0	0	0	0	0
PTPR_B	14	0	0	0	14	0	0	0	0	0	0	0
PGG_nC	8	0	0	0	8	0	0	0	0	0	0	0
PIN_T_PPT	12	0	0	0	12	0	0	0	0	0	0	0
PIN_T_PB_A	3	0	0	1	2	1	0	0	0	0	0	0
PIN_T_PB_B	7	0	0	1	6	1	0	0	0	0	0	0
PITUI	5	2	0	0	3	0	0	2	0	0	0	0
PITAD_STH_DNS_B	1	0	0	0	1	0	0	0	0	0	0	0
PLEX_AD	5	0	0	1	4	1	0	0	0	0	0	0
PLEX_PED_A	6	0	0	2	4	2	0	0	0	0	0	0
PLEX_PED_B	28	3	1	0	24	0	1	3	0	0	0	0
SCHW	18	0	0	0	18	0	0	0	0	0	0	0
SFT_HMPC	16	4	0	0	12	0	0	4	0	0	0	0
SUBEPN_PF	2	1	0	0	1	0	0	1	0	0	0	0
SUBEPN_SPINE	4	0	0	0	4	0	0	0	0	0	0	0
SUBEPN_ST	6	0	0	0	6	0	0	0	0	0	0	0
sum	**2417**	**562**	**199**	**361**	**1747**	**61**	**17**	**264**	**30**	**146**	**28**	**124**

Table 2

n total	n *TERT*	n *EGFR*	n 7/10	double 7/10 + *EGFR*	double 7/10 + *TERT*	double *EGFR* + *TERT*	triple 7/10 + *EGFR* + *TERT*	any double or triple
true positive	363	196	323	29	136	28	124	317
true negative	1674	1870	1835	1872	1863	1873	1873	1862
false positive	199	3	38	1	10	0	0	11
false negative	181	348	221	515	408	516	420	227
sensitivity	**66,7%**	**36,0%**	**59,4%**	**5,3%**	**25,0%**	**5,1%**	**22,8%**	**58,3%**
specificity	**89,4%**	**99,8%**	**98,0%**	**99,9%**	**99,5%**	**100,0%**	**100,0%**	**99,4%**

Figure 1

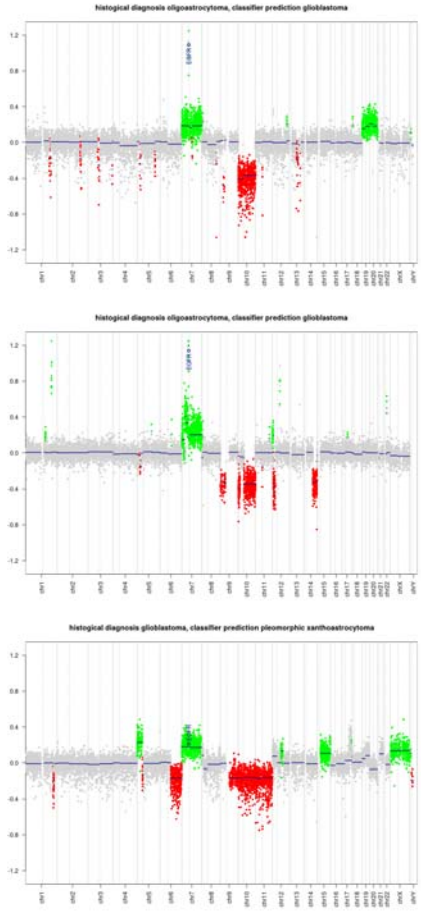
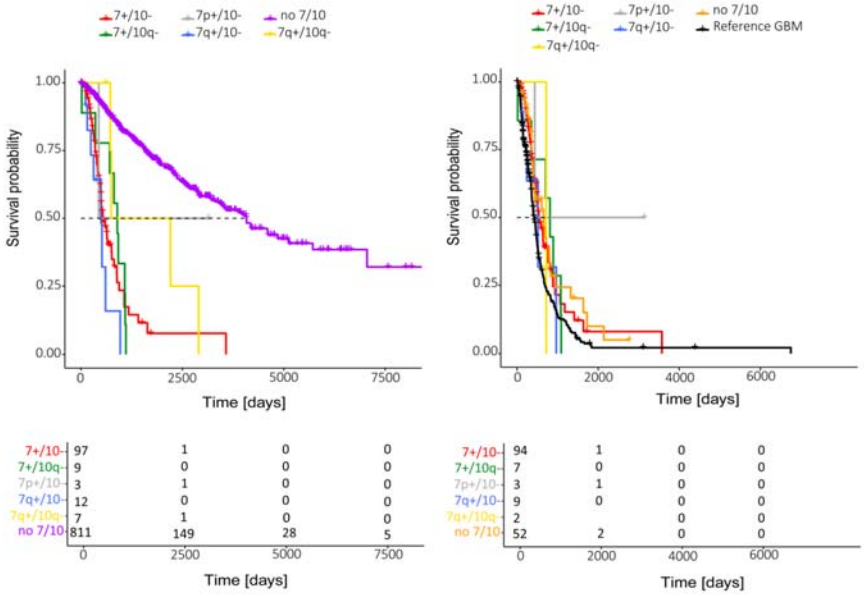

histogical diagnosis oligoastrocytoma, classifier prediction glioblastoma
histogical diagnosis oligoastrocytoma, classifier prediction glioblastoma
histogical diagnosis glioblastoma, classifier prediction pleomorphic xanthoastrocytoma

Figure 2


7+/10-
7+/10q-
7p+/10-
7q+/10-
no 7/10
7q+/10q-
Reference GBM
Survival probability
1.00
0.75
0.50
0.25
0.00
0
2500
5000
7500
2000
4000
6000
Time [days]
7+/10- 97 1 0 0
7+/10q- 9 0 0 0
7p+/10- 3 1 0 0
7q+/10- 12 0 0 0
7q+/10q- 7 1 0 0
no 7/10 811 149 28 5
7+/10- 94 1 0 0
7+/10q- 7 0 0 0
7p+/10- 3 1 0 0
7q+/10- 9 0 0 0
7q+/10q- 2 0 0
no 7/10 52 2 0 0

	methylation class
1	Methylation class (anaplastic) pleomorphic xanthoastrocytoma
2	Methylation class CNS Ewing sarcoma family tumor with CIC alteration
3	Methylation class CNS high grade neuroepithelial tumor with BCOR alteration
4	Methylation class CNS high grade neuroepithelial tumor with MN1 alteration
5	Methylation class CNS neuroblastoma with FOXR2 activation
6	Methylation class Ewing sarcoma
7	Methylation class IDH glioma, subclass 1p/19q codeleted oligodendroglioma
8	Methylation class IDH glioma, subclass astrocytoma
9	Methylation class IDH glioma, subclass high grade astrocytoma
10	Methylation class anaplastic pilocytic astrocytoma
11	Methylation class atypical teratoid/rhabdoid tumor, subclass MYC
12	Methylation class atypical teratoid/rhabdoid tumor, subclass SHH
13	Methylation class atypical teratoid/rhabdoid tumor, subclass TYR
14	Methylation class central neurocytoma
15	Methylation class cerebellar liponeurocytoma
16	Methylation class chordoid glioma of the third ventricle
17	Methylation class chordoma
18	Methylation class craniopharyngioma, adamantinomatous
19	Methylation class craniopharyngioma, papillary
20	Methylation class diffuse leptomeningeal glioneuronal tumor
21	Methylation class diffuse midline glioma H3 K27M mutant
22	Methylation class embryonal tumor with multilayered rosettes
23	Methylation class ependymoma, RELA fusion
24	Methylation class ependymoma, YAP fusion

25 Methylation class ependymoma, myxopapillary

26 Methylation class ependymoma, posterior fossa group A

27 Methylation class ependymoma, posterior fossa group B

28 Methylation class ependymoma, spinal

29 Methylation class esthesioneuroblastoma, subclass A

30 Methylation class esthesioneuroblastoma, subclass B

31 Methylation class glioblastoma, IDH wildtype, H3.3 G34 mutant

32 Methylation class glioblastoma, IDH wildtype, subclass MYCN

33 Methylation class glioblastoma, IDH wildtype, subclass RTK I

34 Methylation class glioblastoma, IDH wildtype, subclass RTK II

35 Methylation class glioblastoma, IDH wildtype, subclass RTK III

36 Methylation class glioblastoma, IDH wildtype, subclass mesenchymal

37 Methylation class glioblastoma, IDH wildtype, subclass midline

38 Methylation class hemangioblastoma

39 Methylation class infantile hemispheric glioma

40 Methylation class low grade glioma, MYB/MYBL1

41 thylation class low grade glioma, desmoplastic infantile astrocytoma / ganglioglio

42 Methylation class low grade glioma, dysembryoplastic neuroepithelial tumor

43 Methylation class low grade glioma, ganglioglioma

44 Methylation class low grade glioma, rosette forming glioneuronal tumor

45 ion class low grade glioma, subclass hemispheric pilocytic astrocytoma and gang

46 Methylation class low grade glioma, subclass midline pilocytic astrocytoma

47 Methylation class low grade glioma, subclass posterior fossa pilocytic astrocytom

48 Methylation class low grade glioma, subependymal giant cell astrocytoma

49 Methylation class lymphoma

50 Methylation class medulloblastoma, WNT

51 SHH_Methylation class medulloblastoma, subclass SHH A (children and adultCH

52 Methylation class medulloblastoma, subclass SHH B (infant)

53 Methylation class medulloblastoma, subclass group 3

54 Methylation class medulloblastoma, subclass group 4

55 Methylation class melanocytoma

56 Methylation class melanoma

57 Methylation class melanotic schwannoma

58 Methylation class meningioma

59 Methylation class papillary tumor of the pineal region group A

60 Methylation class papillary tumor of the pineal region group B

61 Methylation class paraganglioma, spinal non-CIMP

62 Methylation class pineal parenchymal tumor

63 Methylation class pineoblastoma group A / intracranial retinoblastoma

64 Methylation class pineoblastoma group B

65 Methylation class pituicytoma / granular cell tumor / spindle cell oncocytoma

66 Methylation class pituitary adenoma, ACTH

67 Methylation class pituitary adenoma, FSH/LH

68 Methylation class pituitary adenoma, STH densely granulated, group A

69 Methylation class pituitary adenoma, STH densely granulated, group B

70 Methylation class pituitary adenoma, STH sparsely granulated

71 Methylation class pituitary adenoma, TSH

72 PITAD_Methylation class pituitary adenoma, prolactinPRL

73 Methylation class plasmacytoma

74 Methylation class plexus tumor, subclass adult

75	Methylation class plexus tumor, subclass pediatric A
76	Methylation class plexus tumor, subclass pediatric B
78	Methylation class schwannoma
79	Methylation class solitary fibrous tumor / hemangiopericytoma
80	Methylation class subependymoma, posterior fossa
81	Methylation class subependymoma, spinal
82	Methylation class subependymoma, supratentorial

abbrev.	n	$EGFR_{amp}$	$EGFR_{wt}$	$EGFR_{amp}$ %	threshold %
PXA	199	0	199	0.0	50 80
EFT_CIC	85	0	85	0.0	50 80
HGNET_BCOR	62	0	62	0.0	50 80
HGNET_MN1	58	0	58	0.0	50 80
CNS_NB_FOXR2	56	0	56	0.0	50 80
EWS	121	0	121	0.0	50 80
O_IDH	528	1	527	0.2	50 80
A_IDH	640	0	640	0.0	50 80
A_IDH_HG	351	4	347	1.1	50 80
ANA_PA	112	0	112	0.0	50 80
ATRT_MYC	81	0	81	0.0	50 80
ATRT_SHH	109	0	109	0.0	50 80
ATRT_TYR	61	0	61	0.0	50 80
CN	30	0	30	0.0	50 80
LIPN	16	0	16	0.0	50 80
CHGL	17	0	17	0.0	50 80
CHORDM	81	1	80	1.2	50 80
CPH_ADM	41	0	41	0.0	50 80
CPH_PAP	49	0	49	0.0	50 80
DLGNT	29	0	29	0.0	50 80
DMG_K27	292	4	288	1.4	50 80
ETMR	106	0	106	0.0	50 80
EPN_RELA	161	0	161	0.0	50 80
EPN_YAP	15	0	15	0.0	50 80

EPN_MPE	76	1	75	1.3	50 80
EPN_PF_A	367	0	367	0.0	50 80
EPN_PF_B	95	0	95	0.0	50 80
EPN_SPINE	59	0	59	0.0	50 80
ENB_A	23	0	23	0.0	50 80
ENB_B	22	0	22	0.0	50 80
GBM_G34	105	4	101	3.8	50 80
GBM_MYCN	47	12	35	25.5	50 80
GBM_RTK_I	350	73	277	20.9	50 80
GBM_RTK_II	873	599	274	68.6	50 80
GBM_RTK_III	42	13	29	31.0	50 80
GBM_MES	705	197	508	27.9	50 80
GBM_MID	125	0	125	0.0	50 80
HMB	57	0	57	0.0	50 80
IHG	37	0	37	0.0	50 80
LGG_MYB	88	0	88	0.0	50 80
LGG_DIG_DIA	18	0	18	0.0	50 80
LGG_DNT	136	0	136	0.0	50 80
LGG_GG	75	0	75	0.0	50 80
LGG_RGNT	26	0	26	0.0	50 80
LGG_PA_GG_ST	120	0	120	0.0	50 80
LGG_PA_MID	115	0	115	0.0	50 80
LGG_PA_PF	279	0	279	0.0	50 80
LGG_SEGA	29	0	29	0.0	50 80
LYMPHO	39	0	39	0.0	50 80

MB_WNT	125	0	125	0.0	50 80
MB_SHH_CHL_AD	253	0	253	0.0	50 80
MB_SHH_INF	142	0	142	0.0	50 80
MB_G3	257	0	257	0.0	50 80
MB_G4	513	0	513	0.0	50 80
MELCYT	56	1	55	1.8	50 80
MELAN	87	0	87	0.0	50 80
SCHW_MEL	13	0	13	0.0	50 80
MNG	1058	1	1057	0.1	50 80
PTPR_A	11	0	11	0.0	50 80
PTPR_B	32	0	32	0.0	50 80
PGG_nC	24	0	24	0.0	50 80
PIN_T_PPT	37	0	37	0.0	50 80
PIN_T_PB_A	24	0	24	0.0	50 80
PIN_T_PB_B	43	0	43	0.0	50 80
PITUI	55	0	55	0.0	50 80
PITAD_ACTH	48	0	48	0.0	50 80
PITAD_FSH_LH	53	0	53	0.0	50 80
?ITAD_STH_DNS_A	15	0	15	0.0	50 80
?ITAD_STH_DNS_B	27	0	27	0.0	50 80
PITAD_STH_SPA	28	0	28	0.0	50 80
PITAD_TSH	25	0	25	0.0	50 80
PITAD_PRL	17	1	16	5.9	50 80
PLASMA	10	0	10	0.0	50 80
PLEX_AD	36	0	36	0.0	50 80

PLEX_PED_A	32	1	31	3.1	50 80
PLEX_PED_B	307	7	300	2.3	50 80
SCHW	155	1	154	0.6	50 80
SFT_HMPC	37	0	37	0.0	50 80
SUBEPN_PF	54	1	53	1.9	50 80
SUBEPN_SPINE	19	0	19	0.0	50 80
SUBEPN_ST	25	0	25	0.0	50 80

$7p_{wt}\_7q_{wt}\_10p_{wt}\_10q_{wt}$	$7p_{wt}\_7q_{wt}\_10p_{wt}\_10q_{loss}$	$7p_{wt}\_7q_{wt}\_10p_{loss}\_10q_{wt}$
90	6	5
93	4	5
71	1	3
72	1	3
57	1	0
57	1	0
44	2	0
44	2	0
39	10	0
45	4	1
96	3	3
96	3	3
460	4	2
481	1	2
416	22	2
490	8	2
133	79	4
205	38	7
70	7	14
79	4	13
76	0	3
77	0	2
98	2	0
98	2	0
57	0	0
57	0	0
30	0	0
30	0	0
14	1	0
14	1	0
17	0	0
17	0	0
27	0	1
27	2	1
36	0	1
36	0	1
40	1	2
40	1	2
18	0	0
18	0	0
180	40	3
199	27	4
76	0	6
78	0	6
128	1	2
128	1	2
15	0	0
15	0	0

23	0	0
23	0	0
317	10	4
319	8	4
6	0	2
6	0	2
28	0	1
28	0	1
2	0	0
2	0	0
2	0	0
2	0	0
36	21	3
57	5	4
11	6	0
15	4	0
4	4	0
4	5	1
6	3	2
9	3	2
13	4	0
15	3	1
39	7	5
42	7	5
68	9	4
81	6	3
56	0	0
56	0	0
32	0	1
32	0	1
87	0	1
87	0	1
15	0	2
15	0	2
120	0	0
120	0	0
53	3	1
55	1	1
24	0	0
25	0	0
84	0	1
85	0	0
106	0	1
107	0	0
244	1	1
245	1	1
29	0	0
29	0	0
27	0	1
30	0	0

103	3	3
105	1	3
184	39	1
200	25	1
106	9	2
108	7	2
76	21	1
100	7	1
248	13	6
270	3	7
53	2	0
53	2	0
27	4	5
30	5	6
8	0	0
8	0	0
828	44	18
841	37	17
1	0	0
2	0	0
1	0	0
1	0	0
24	0	0
24	0	0
29	0	1
29	0	1
19	1	0
20	0	0
16	1	0
18	0	0
50	1	0
51	0	0
32	0	0
32	0	0
51	1	0
52	0	0
3	0	0
3	0	0
21	3	0
22	2	0
28	0	0
28	0	0
9	1	0
9	1	0
1	1	0
1	1	0
6	0	0
6	0	0
1	0	0
1	0	0

18	0	0
18	0	0
172	16	18
177	14	19
146	0	2
147	0	1
37	0	0
37	0	0
50	1	1
51	0	1
18	0	1
18	0	1
25	0	0
25	0	0

$7p_{wt}\_7q_{wt}\_10p_{loss}\_10q_{loss}$	$7p_{wt}\_7q_{gain}\_10p_{wt}\_10q_{wt}$	$7p_{wt}\_7q_{gain}\_10p_{wt}\_10q_{loss}$
32	2	0
31	3	0
3	1	0
4	1	0
1	0	0
1	0	0
10	0	0
10	0	0
2	0	0
1	0	0
11	0	0
11	0	0
12	23	0
12	7	0
0	131	5
0	80	1
32	33	16
31	20	5
6	5	0
6	3	0
1	0	0
1	0	0
5	0	0
5	0	0
0	0	0
0	0	0
0	0	0
0	0	0
1	0	0
1	0	0
0	0	0
0	0	0
19	1	1
18	1	0
0	0	0
0	0	0
3	0	0
3	0	0
2	4	0
2	4	0
26	6	3
25	6	3
7	0	0
7	0	0
16	0	0
16	0	0
0	0	0
0	0	0

17	0	0
17	0	0
7	1	0
7	1	0
18	0	0
18	0	0
5	0	0
5	0	0
9	0	0
9	0	0
16	0	0
16	0	0
4	5	1
2	5	0
9	3	2
9	4	0
39	0	0
42	2	0
82	0	2
98	1	2
6	0	0
5	0	0
108	6	3
114	7	2
19	5	0
17	3	0
1	0	0
1	0	0
2	0	0
2	0	0
0	0	0
0	0	0
1	0	0
1	0	0
3	1	0
3	1	0
0	1	0
0	1	0
0	1	0
0	0	0
2	1	0
2	1	0
0	0	0
0	0	0
1	1	0
1	2	0
0	0	0
0	0	0
2	3	0
1	2	0

9	1	0
9	1	0
14	4	1
12	5	1
13	0	0
13	0	0
38	10	13
38	14	2
15	24	3
16	17	0
0	0	0
0	0	0
12	2	2
13	2	2
1	0	0
1	0	0
103	20	3
102	18	2
6	0	0
6	0	0
30	0	0
30	0	0
0	0	0
0	0	0
4	0	0
4	0	0
1	0	0
1	0	0
3	2	1
4	1	1
1	1	0
1	1	0
10	0	0
10	0	0
0	0	0
0	0	0
1	0	0
1	0	0
0	0	0
0	0	0
0	0	0
0	0	0
4	0	0
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0	0	0
1	0	0
1	0	0
2	1	0
2	1	0

2	0	0
2	0	0
44	2	0
44	2	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

$7p_{wt}\_7q_{gain}\_10p_{loss}\_10q_{wt}$	$7p_{wt}\_7q_{gain}\_10p_{loss}\_10q_{loss}$	$7p_{gain}\_7q_{wt}\_10p_{wt}\_10q_{wt}$
1	3	3
0	3	4
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	2
0	0	2
1	0	2
1	0	1
1	0	8
1	0	6
1	5	5
0	2	5
0	1	2
0	0	2
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
1	0	1
1	0	2
0	0	0
0	0	1
0	0	1
0	0	2
0	0	0
0	0	0
0	0	10
0	0	8
0	0	3
0	0	2
0	0	0
0	0	0
0	0	0
0	0	0

0	0	1
0	0	1
0	0	1
0	0	1
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
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0	0	0
0	0	0
0	3	4
0	3	4
1	0	2
0	0	3
0	8	2
0	9	2
1	35	0
0	53	1
1	0	2
0	0	3
3	24	8
1	32	9
0	0	6
0	0	7
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	1
0	0	1
0	0	0
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0	0	2
0	0	2
0	0	0
0	0	0
0	0	2
0	0	1
0	0	0
0	0	0
0	0	0
0	0	0

0	0	0
0	0	0
0	1	1
0	1	1
0	0	1
0	0	1
1	3	3
1	4	3
1	3	7
0	2	8
0	0	0
0	0	0
3	4	4
1	2	4
0	1	0
0	1	0
0	3	2
0	3	1
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	1
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0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

0	0	0
0	0	0
1	2	6
0	2	7
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

$7p_{gain}\_7q_{wt}\_10p_{wt}\_10q_{loss}$	$7p_{gain}\_7q_{wt}\_10p_{loss}\_10q_{wt}$	$7p_{gain}\_7q_{wt}\_10p_{loss}\_10q_{loss}$
0	0	1
0	0	1
0	1	1
0	1	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
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6	0	0
3	0	0
0	1	0
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0	0	0
0	0	0
2	2	0
1	1	0
0	2	0
0	2	0
0	0	0
0	0	0
0	0	0
0	0	0

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0	0	1
0	0	0
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0	0	0
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0	0	1
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0	0	1
0	0	1
2	0	1
2	0	3
0	0	3
0	0	3
2	1	12
3	0	18
3	0	30
1	0	35
0	0	1
0	0	1
1	1	25
1	1	32
3	1	2
0	0	2
0	0	0
0	0	0
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0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
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0	0	0
0	0	0
2	0	0
0	0	0
0	1	1
0	1	1
0	0	0
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0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
1	0	0
1	0	0

0	0	0
0	0	0
5	0	7
4	0	4
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

$7p_{gain}\_7q_{gain}\_10p_{wt}\_10q_{wt}$	$7p_{gain}\_7q_{gain}\_10p_{wt}\_10q_{loss}$	$7p_{gain}\_7q_{gain}\_10p_{loss}\_10q_{wt}$
38	0	2
37	0	3
2	0	1
2	0	0
2	0	1
2	0	1
1	0	0
1	0	0
4	1	0
5	0	0
5	0	1
5	0	1
22	1	0
21	1	0
53	2	0
52	0	0
25	10	1
30	3	1
5	0	0
4	0	0
1	0	0
1	0	0
2	0	0
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5	0	0
5	0	0
16	1	0
15	0	0
11	1	0
10	1	0
10	0	2
11	0	1
0	0	0
0	0	0

30	0	0
30	0	0
23	2	0
23	2	0
60	0	3
60	0	3
23	0	0
23	0	0
2	1	0
2	1	0
1	0	0
1	0	0
11	11	0
14	5	1
7	1	0
6	1	0
36	28	4
37	25	4
35	29	11
45	23	8
5	2	1
7	0	0
43	16	8
43	15	10
7	0	0
5	0	0
0	0	0
0	0	0
2	0	0
2	0	0
0	0	0
0	0	0
0	0	0
0	0	0
10	0	0
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28	0	0
28	0	0
8	0	0
8	0	0
29	0	0
28	0	0
0	0	0
0	0	0
6	0	0
6	0	0

5	0	0
5	0	0
7	1	0
7	0	0
10	1	0
11	0	0
60	10	3
66	4	2
127	18	6
134	8	6
1	0	0
1	0	0
13	4	2
13	3	1
3	0	0
3	0	0
33	1	0
33	1	0
3	0	0
2	0	0
1	0	0
1	0	0
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0	0	0
0	0	0
9	0	0
9	0	0
14	0	1
15	0	0
3	0	0
3	0	0
21	0	0
21	0	0

7	0	0
7	0	0
23	1	2
24	1	1
7	0	0
7	0	0
0	0	0
0	0	0
2	0	0
2	0	0
0	0	0
0	0	0
0	0	0
0	0	0

$7p_{gain}\_7q_{gain}\_10p_{loss}\_10q_{loss}$
16
15
1
1
0
0
1
1
0
0
0
0
1
1
0
0
1
1
1
1
0
0
2
2
0
0
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0
0
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0
18
18
0
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0
0
0
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3
3
0
0
2
2
0
0

4
4
2
2
6
6
2
2
8
8
2
2
3
0
2
2
210
198
634
592
7
7
408
384
1
1
0
0
0
0
0
0
0
0
0
0
2
2
0
0
2
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0

1
1
0
0
0
0
16
15
40
40
0
0
3
3
0
0
1
1
1
1
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0
0
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0
5
5
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1
1
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0
2
2
0
0
0
0
2
2
0
0
0
0
10
10

5
5
8
8
0
0
0
0
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0